

## SEQUENCE LISTING



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<120> Single Promoter System for Making siRNA Expression  
Cassettes and Expression Libraries Using a Polymerase  
Primer Hairpin Linker

<130> 016556-003210US

<140> US 10/628,587  
<141> 2003-07-23

<150> US 60/399,040  
<151> 2002-07-24

<160> 34

<170> PatentIn Ver. 2.1

<210> 1  
<211> 28  
<212> DNA  
<213> Artificial Sequence

<220>  
<223> Description of Artificial Sequence:upstream primer  
Hind III U6-265 modified to contain a Hind III  
site outside the 5' end of the U6 promoter

<400> 1  
tgctaagctt aaggctgggc aggaagag

28

<210> 2  
<211> 26  
<212> DNA  
<213> Artificial Sequence

<220>  
<223> Description of Artificial Sequence:downstream  
primer S-U6-20 modified to contain a Sph I  
restriction site at the 3' end of the U6 promoter

<400> 2  
atcggcatgc agatatataa agccaa

26

<210> 3  
<211> 43  
<212> DNA  
<213> Artificial Sequence

<220>  
<223> Description of Artificial Sequence:chemically synthesized self-priming oligonucleotide siRNA-LIBh with complement of pol III promoter type III termination signal, randomized "sense" coding sequence for hairpin siRNA and linker and primer for synthesis of "antisense" strand

<220>  
<221> modified\_base  
<222> (1)  
<223> n = 5' phosphorylated c

<220>  
<221> modified\_base  
<222> (16)..(33)  
<223> n = g, a, c or t

<400> 3  
ngaccactct aaaaannnnn nnnnnnnnnn nnngcgttcg cgcc

43

<210> 4  
<211> 19  
<212> DNA  
<213> Artificial Sequence

<220>  
<223> Description of Artificial Sequence:chemically synthesized universal oligonucleotide Univ-1h (Sph I)

<400> 4  
tttttagagt ggtcgcattg

19

<210> 5  
<211> 19  
<212> DNA  
<213> Artificial Sequence

<220>  
<223> Description of Artificial Sequence:chemically synthesized universal oligonucleotide Univ-2h (Bam HI)

<220>  
<221> modified\_base  
<222> (1)  
<223> n = 5' phosphorylated g

<400> 5  
natccgacct ctctaaaaaa

19

<210> 6  
<211> 43  
<212> DNA  
<213> Artificial Sequence

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<220>
<223> Description of Artificial Sequence:chemically
      synthesized oligonucleotide siRNAh-lucB

<220>
<221> modified_base
<222> (1)
<223> n = 5' phosphorylated c

<400> 6
ngaccactct aaaaagtgcg ctgctggtgc caacccttcg ggg          43

<210> 7
<211> 43
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence:chemically
      synthesized oligonucleotide siRNAh-SCRAMBLE

<220>
<221> modified_base
<222> (1)
<223> n = 5' phosphorylated c

<400> 7
ngaccactct aaaaagcgcg cttagtagga ttccgcgttcg cgcc         43

<210> 8
<211> 54
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence:downstream
      primer S-U6-TET-o modified to contain a Sph I
      restriction site at the 3' end of the U6 promoter
      and incorporating tetracycline operator sequence

<400> 8
atcggcatgc agatatataa ctcttatcaat gatagagttac tttcaagtta cgg          54

<210> 9
<211> 97
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence:self-priming
      oligonucleotide HpLib containing spacer, Asc I
      restriction site, part of XmaI site, spacer,
      complement to transcription terminator, randomized
      siRNA coding sequence and polymerase primer hairpin
      linker

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<220>
<221> modified_base
<222> (31)..(49)
<223> n = g, a, c or t, randomized siRNA coding sequence

<400> 9
ttcttagaggc gcgcggggcc gccaaaaaag nnnnnnnnnn nnnnnnnnnnc ttcaagcgaa 60
gagcgcctcc ggtaacggag gcgcgttccg aagagag 97

<210> 10
<211> 32
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence:2nd Strand
      Primer

<400> 10
cccccccccc cccccccggg ccgcgggg ag 32

<210> 11
<211> 42
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence:self-priming
      oligonucleotide with 5' leader sequence,
      randomized siRNA coding sequence and polymerase
      primer hairpin linker sequence

<220>
<221> modified_base
<222> (7)..(10)
<223> n = g, a, c or t

<220>
<221> modified_base
<222> (16)..(32)
<223> n = g, a, c or t, coding sequence for "sense"
      strand of siRNA

<400> 11
ggccgcnnnn aaaaannnnn nnnnnnnnnn nngggttcgc cc 42

<210> 12
<211> 74
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence:self-priming
      oligonucleotide after primer extension of SEQ ID
      NO:11 to generate sequence complementary to 5'
      leader sequence and randomized siRNA coding
      region "sense" strand to form a stem-loop structure

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<220>
<221> modified_base
<222> (7)..(10)
<223> n = g, a, c or t, complementary to n = g, a, c or
      t at positions 68-65

<220>
<221> modified_base
<222> (16)..(32)
<223> n = g, a, c or t, complementary to n = g, a, c or
      t at positions 59-43

<220>
<221> modified_base
<222> (43)..(59)
<223> n = g, a, c or t, complementary to n = g, a, c or
      t at positions 32-16

<220>
<221> modified_base
<222> (65)..(68)
<223> n = g, a, c or t, complementary to n = g, a, c or
      t at positions 10-7

<400> 12
ggccgcnnnn aaaaannnnn nnnnnnnnnn nngggttcgc ccnnnnnnnn nnnnnnnnnnt 60
ttttnnnnngc ggcc                                         74

<210> 13
<211> 11
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence:5' blocking
      primer

<220>
<221> modified_base
<222> (6)..(9)
<223> n = g, a, c or t, complementary to n = g, a, c or t
      at positions 10-7 of SEQ ID NO:12

<400> 13
tttttnnnnng c                                         11

<210> 14
<211> 19
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence:3' blocking
      primer

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<220>
<221> modified_base
<222> (11)..(14)
<223> n = g, a, c or t, complementary to n = g, a, c or t
      at positions 68-65 of SEQ ID NO:12

<400> 14
cgcgccgc nnnnaaaa 19

<210> 15
<211> 74
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence:completed
      expression cassette sequence with synthesized
      segment complementary to single stranded region
      between blocking primers

<220>
<221> modified_base
<222> (11)..(14)
<223> n = g, a, c or t, complementary to positions 72-69

<220>
<221> modified_base
<222> (20)..(36)
<223> n = g, a, c or t, complementary to positions 63-47

<220>
<221> modified_base
<222> (47)..(63)
<223> n = g, a, c or t, complementary to positions 36-20

<220>
<221> modified_base
<222> (69)..(72)
<223> n = g, a, c or t, complementary to positions 14-11

<400> 15
cgcgccgc nnnnaaaaan nnnnnnnnnn nnnnnnggc gaaccnnnn nnnnnnnnnn 60
nnntttttnn nngc 74

<210> 16
<211> 137
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence:self-priming
      oligonucleotide HPLib after primer extension with
      reverse transcriptase (RT)

<220>
<221> modified_base
<222> (31)..(49)
<223> n = g, a, c or t, complementary to positions 107-89

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<220>
<221> modified_base
<222> (89)..(107)
<223> n = g, a, c or t, complementary to positions 49-31

<400> 16
ttcttagaggc gcgcggggcc gccaaaaaaag nnnnnnnnnn nnnnnnnnnnc ttcaagcgaa 60
gagttacgga ggcgtcttc gaagagagnn nnnnnnnnnn nnnnnnnnctt ttttggcggc 120
ccggcgccc tctagaa 137

<210> 17
<211> 115
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence:self-priming
      oligonucleotide HpLib after primer extension with
      reverse transcriptase (RT) and AscI digestion to
      yield recessed 3' end

<220>
<221> modified_base
<222> (22)..(40)
<223> n = g, a, c or t, complementary to positions 98-80

<220>
<221> modified_base
<222> (80)..(98)
<223> n = g, a, c or t, complementary to positions 40-22

<400> 17
cgcgccgggc cgccaaaaaa gnnnnnnnnn nnnnnnnnnn cttcaagcgaa agagttacgg 60
aggcgctctt cgaagagagnn nnnnnnnnnn nnnnnnnnctt ttttggcggc cccgg 115

<210> 18
<211> 118
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence:self-priming
      oligonucleotide HpLib after primer extension with
      reverse transcriptase (RT), AscI digestion to
      yield recessed 3' end and addition of oligo(dG)
      homopolymer tail using terminal transferase

<220>
<221> modified_base
<222> (22)..(40)
<223> n = g, a, c or t, complementary to positions 98-80

<220>
<221> modified_base
<222> (80)..(98)
<223> n = g, a, c or t, complementary to positions 40-22

<400> 18
cgcgccgggc cgccaaaaaa gnnnnnnnnn nnnnnnnnnn cttcaagcgaa agagttacgg 60

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aggcgctcgg cgaagagagn nnnnnnnnnn nnnnnnnnct ttttggcg  cccggggg      118
```

```
<210> 19
<211> 126
<212> DNA
<213> Artificial Sequence

<223> Description of Artificial Sequence:self-priming
      oligonucleotide HpLib after primer extension with
      reverse transcriptase (RT) and AsCI digestion to
      yield recessed 3' end and addition of oligo(dG)
      homopolymer tail using terminal transferase and
      ligation of AsCI linkers using T4 DNA ligase

<220>
<221> modified_base
<222> (30)..(48)
<223> n = g, a, c or t, complementary to positions 106-88

<220>
<221> modified_base
<222> (88)..(106)
<223> n = g, a, c or t, complementary to positions 48-30

<400> 19
ggcgcgccccg cgccgggcccg ccaaaaaagn nnnnnnnnnn nnnnnnnnct tcaagcgaag 60
agttagggag gcgcttcg aagagagnnn nnnnnnnnnn nnnnnncttt tttggcgccc 120
                                         126
cgggggg

<210> 20
<211> 117
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence:self-priming
      oligonucleotide HpLib SEQ ID NO:19 after digestion
      with AsCI and XmaI to yield distinct 5' overhang

<220>
<221> modified_base
<222> (28)..(46)
<223> n = g, a, c or t, complementary to positions 104-86

<220>
<221> modified_base
<222> (86)..(104)
<223> n = g, a, c or t, complementary to positions 46-28

<400> 20
cgcgccccgccc ccggggccgc aaaaaagnnn nnnnnnnnnn nnnnnnncttc aagcgaagag 60
ttacggaggc gcttcgaa gagagnnnnn nnnnnnnnnn nnnnctttt tggcgcc 117

<210> 21
<211> 126
<212> DNA
<213> Artificial Sequence
```

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<220>
<223> Description of Artificial Sequence:self-priming
      oligonucleotide HpLib complement of SEQ ID NO:19
      after digestion with AscI and XmaI to yield
      distinct 5' overhang

<220>
<221> modified_base
<222> (18)..(36)
<223> n = g, a, c or t, complementary to positions 103-85

<220>
<221> modified_base
<222> (85)..(103)
<223> n = g, a, c or t, complementary to positions 36-18

<400> 21
ccgggcccgc aaaaaagnnn nnnnnnnnnn nnnnnnctct cttcgaagag cgccctccgta 60
accggaggcg ctcttcgctt gaagnnnnnn nnnnnnnnnn nnncctttt ggcggccccgg 120
                                         126
cgcggg

<210> 22
<211> 124
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence:self-priming
      oligonucleotide HpLib, SEQ ID NO:20 AscI/XmaI
      digested product after ligation into vector
      bearing U6 snRNA promoter

<220>
<221> modified_base
<222> (30)..(48)
<223> n = g, a, c or t, complementary to positions 106-88

<220>
<221> modified_base
<222> (88)..(106)
<223> n = g, a, c or t, complementary to positions 48-30

<400> 22
ggcgcgccccg cgccggggccg ccaaaaaagn nnnnnnnnnn nnnnnnnnct tcaagcgaag 60
attacggag gcgctttcg aagagagnnn nnnnnnnnnn nnnnnncttt tttggcgccc 120
                                         124
cggg

<210> 23
<211> 52
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence:SEQ ID NO:22
      after disgetion with SapI and elimination of
      majority of polymerase primer hairpin linker

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<220>
<221> modified_base
<222> (30)..(48)
<223> n = g, a, c or t, complementary to positions 26-8
      of SEQ ID NO:24 and positions 26-8 of SEQ ID NO:25

<400> 23
ggcgcgccccg cgccgggcccg ccaaaaaagn nnnnnnnnnn nnnnnnnnct tc      52

<210> 24
<211> 44
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence:SEQ ID NO:22
      after disgetion with SapI and elimination of
      majority of polymerase primer hairpin linker

<220>
<221> modified_base
<222> (8)..(26)
<223> n = g, a, c or t, complementary to positions 48-30
      of SEQ ID NO:23 and positions 37-19 of SEQ ID NO:26

<400> 24
aagagagnnn nnnnnnnnnn nnnnnnctt tttggcggcc cggg      44

<210> 25
<211> 55
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence:complement of
      SEQ ID NO:22 after disgetion with SapI and
      elimination of majority of polymerase primer hairpin
      linker

<220>
<221> modified_base
<222> (8)..(26)
<223> n = g, a, c or t, complementary to positions 48-30
      of SEQ ID NO:23 and positions 37-19 of SEQ ID NO:26

<400> 25
cttgaagnnn nnnnnnnnnn nnnnnnctt tttggcggcc cggcgcgggc gcgcc      55

<210> 26
<211> 41
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence:complement of
      SEQ ID NO:22 after disgetion with SapI and
      elimination of majority of polymerase primer hairpin
      linker

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```
<220>
<221> modified_base
<222> (19)..(37)
<223> n = g, a, c or t, complementary to positions 26-8
      of SEQ ID NO:24 and positions 26-8 of SEQ ID NO:25
```

```
<400> 26
cccgccccgc caaaaaagnn nnnnnnnnnn nnnnnnnnctc t
```

41

```
<210> 27
<211> 96
<212> DNA
<213> Artificial Sequence
```

```
<220>
<223> Description of Artificial Sequence:intramolecular
      re-ligation of SEQ ID NOS:23 and 24 forming coding
      region for loop expressed as component of hairpin
      siRNA
```

```
<220>
<221> modified_base
<222> (30)..(48)
<223> n = g, a, c or t, complementary to positions 78-60
```

```
<220>
<221> modified_base
<222> (60)..(78)
<223> n = g, a, c or t, complementary to positions 48-30
```

```
<400> 27
ggcgcgccccg cgccggggccg caaaaaagnn nnnnnnnnnn nnnnnnnnct tcaagagagn 60
nnnnnnnnnnn nnnnnnnnct ttttggcgg cccggg 96
```

```
<210> 28
<211> 15
<212> DNA
<213> Artificial Sequence
```

```
<220>
<223> Description of Artificial Sequence:5' leader
      sequence, first segment of self-priming
      oligonucleotide
```

```
<220>
<221> modified_base
<222> (7)..(10)
<223> n = g, a, c or t
```

```
<400> 28
ggccgcnnnn aaaaa
```

15

```
<210> 29
<211> 10
<212> DNA
<213> Artificial Sequence
```

<220>  
<223> Description of Artificial Sequence:remainder of 5'  
leader sequence before complement of transcription  
termination sequence at 3' end of leader sequence

<220>  
<221> modified\_base  
<222> (7)..(10)  
<223> n = g, a, c or t

<400> 29  
ggccgcnnnn

10

<210> 30  
<211> 10  
<212> DNA  
<213> Artificial Sequence

<220>  
<223> Description of Artificial Sequence:polymerase  
primer hairpin linker, third segment of self-priming  
oligonucleotide

<400> 30  
gggttcgccc

10

<210> 31  
<211> 15  
<212> DNA  
<213> Artificial Sequence

<220>  
<223> Description of Artificial Sequence:segment  
complementary to 5' leader sequence

<220>  
<221> modified\_base  
<222> (6)..(9)  
<223> n = g, a, c or t

<400> 31  
tttttnnnnng cggcc

15

<210> 32  
<211> 10  
<212> DNA  
<213> Artificial Sequence

<220>  
<223> Description of Artificial Sequence:linker and  
primer for synthesis of "antisense" strand of  
hairpin siRNA

<400> 32  
gcgttcgcgc

10

<210> 33  
<211> 22  
<212> DNA  
<213> Artificial Sequence  
  
<220>  
<223> Description of Artificial Sequence:1-N segment of  
polymerase primer hairpin linker

<400> 33  
cttcaagcga agagcgcctc cg

22

<210> 34  
<211> 22  
<212> DNA  
<213> Artificial Sequence  
  
<220>  
<223> Description of Artificial Sequence:3-N segment of  
polymerase primer hairpin linker

<400> 34  
cggaggcgct cttcgaagag ag

22